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Docket No. CDS-226

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Thomas J. Cummins, et al.

Serial No. : 09/675,828 Art Unit: 1637

Filed : September 29, 2000 Examiner: T.E.Strzelecka

For : DIAGNOSTIC COMPOSITIONS, ELEMENTS, METHODS AND TEST KITS FOR AMPLIFICATION AND DETECTION OF TWO OR MORE DNA's USING PRIMERS HAVING MATCHED MELTING TEMPERATURES

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on

June 29, 2004
(Date)

Catherine Kurtz Gowen
Name of applicant, assignee, or Registered Representative

CKGowen
(Signature)

June 24, 2004
(Date of Signature)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF SUSAN MELISSA WERNER PURSUANT TO 37 CFR 1.182

Dear Sir:

SUSAN M. WERNER DECLARES AS FOLLOWS:

1. I, SUSAN M. WERNER, am Regulatory Affairs Manager at Ortho-Clinical Diagnostics, Inc. and a co-inventor of the claims of the captioned application.

2. On the date the original Declaration was signed by me on May 14, 1993, I was known as Susan M. Atwood.

3. I was married on September 2, 2000, and have thereupon changed my name to Susan M. Werner.

4. Attorney of record Catherine Kurtz Gowen was first notified of my name change on or about May 25, 2004.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Susan M. Atwood
Susan M. Atwood

Susan M. Werner
Susan M. Werner

6/24/04
Date

6/24/04
Date

Docket No. CDS-226

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Thomas J. Cummins, et al.

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June 29, 2004
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Catherine Kurtz Gowen
Name of applicant, assignee, or Registered Representative

CKGowen
(Signature)

June 29, 2004
(Date of Signature)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.131

Dear Sir:

I, Susan M. Werner, depose and state:

1. I am coinventor of claims 28, 29, 33 and 36-40 of the above-identified patent application.

2. Prior to December 20, 1990, in collaboration with co-inventors Thomas J. Cummins, Lynn Bergemeyer, John

Bruce Findlay, John W.H. Sutherland and JoAnne H. Kerschner, I conceived and reduced to practice in this country, methods for the simultaneous amplification and detection for 2 or more target DNAs as described and claimed in our application. The following EXHIBITS A, B, C and D evidence the claimed invention:

A. A copy of my Laboratory Notebook No. BB1001, pages 89-90 and pages 95-97, (dated before December 20, 1990), provided as Exhibit A. The notebook entry shows the amplification and detection of 2 targets separated from each other along opposing strands by from 90-400 nucleotides, wherein the amplification uses 4 primers having Tms between 65°-74°C or 67°-74°C, wherein the primers are within about 2°C of each other, wherein priming and primer extension take place at 70°C (all the above documented specifically at pages 89-90 and 95-97) and wherein the primers have nucleotide lengths which differ from each other by no more than 5 nucleotides.

B. A copy of my Laboratory Notebook No. BB1001, pages 179-180 (dated after December 20, 1990 but before July 1992), provided as Exhibit B. Confirmation that the primers nucleotide lengths differed from each other by no more than 5 nucleotides is found at pages 179-180.

C. A copy of Kodak Technical Report (hereafter "TR") RPT. ACC. NO. 249038H co-authored by coinventor John W.H. Sutherland, (dated before December 20, 1990), title page and page 18, provided as Exhibit C. The title page provides an Abstract which summarizes the invention with respect to primer Tms and provides a working formula for calculating primer Tm as:

$$Tm(°C) = 66.5 + 0.36\ (\%G+C) - 384/N,$$

which calculation was used for obtaining primer Tms for the work done as documented in EXHIBIT A. EXHIBIT C goes on to state at page 18 that following the additional research described in the text of this TR, a modified regression equation for calculating primer Tm was derived as:

$$T_{m\ calc} = 67.5 + 0.34(\%G+C) - 395/N,$$

which it is said at the bottom of page 18 "differed only slightly from" the prior equation. This latter formula is identical to the formula provided in the patent disclosure at page 12, line 30 and page 26, line 30, as:

$Tm(°C) = 67.5 + 0.34(G+C) - 395/N,$

and which formula appears in the pending claims.

D. A copy of co-inventor Thomas J. Cummins's Laboratory Notebook No. AA9223 at pages 13-14 (dated before December 20, 1990) provided as EXHIBIT D. The entry on these pages is a record of the conception of use of matched primer Tms in use of PCR that employs combined annealing and extension temperatures.

3. The date deleted from Exhibit A is prior to December 20, 2000 and July 1992.

4. The date deleted from Exhibit B is prior to July 1992.

5. The date deleted from Exhibit C is prior to December 20, 2000 and July 1992.

6. The date deleted from Exhibit D is prior to December 20, 2000 and July 1992.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Susan M. Werner

Susan M. Werner

Date 6/24/04

EXHIBIT A

Notebook No. BB1001

RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

Date.

Problem: HIV PCR

Objective: To determine if new batch of dNTP's functions under HIV assay conditions.

REAGENTS:

10X HI SALT PCR BUFFER - pH 8.0 (100mM Mg++, 100mM Tris) batch 006 prepared

dNTP'S - Sigma batch 006 100µM prepared by NG

PRIMER 1 - EKGAG-F AA9316/106 10µM
PRIMER 2 - EKGAG-R AA9316/106 10µM
PRIMER 3 - EKENV-F AA9316/107 10µM
PRIMER 4 - EKENV-R AA9316/120 10µM

KODAK TAQ - (4 units/µl) EK004 prepared by SA

GENOMIC - H9 and 8E5 cell lysate #0018E5

REAGENT	AMT/TUBE (µl)	# OF TUBES	STOCK AMT (µl)
10X PCR BUFFER	25	9	225
dNTP's	15	9	135
PRIMER 1	25	9	225
PRIMER 2	25	9	225
PRIMER 3	25	9	225
PRIMER 4	25	9	225
GLYCEROL (7.5%)	18.75	9	168.75
KTAQ (4 units/µl)	10	9	90
GENOMIC	25	9	225
TE BUFFER	56.25	9	506.25
		TOTAL	2250

FILL POUCHES WITH 250µl

Processor #4 profile: 90°C preheat - 10 seconds
96°C for 30 seconds
70°C for 60 seconds
for 40 cycles

~~Blood pouches # 194~~

KP 15226-5/86 I. P. S.

Signature Susan M Atwood

The foregoing disclosed to me on___,

Witness

RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

Notebook No. BB1001

Date

Problem: page 89 cont'd

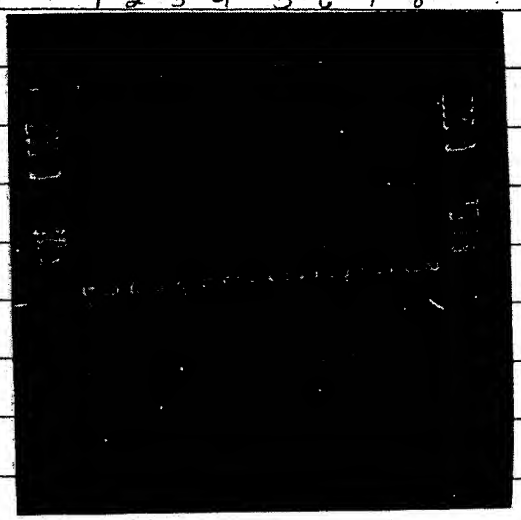
8 blank pouches were run:

position	serial #	position	serial #
1	194974	5	194964
2	194966	6	194973
3	194965	7	194968
4	194975	8	194971

Results

Sample #

1 2 3 4 5 6 7 8



Both EKCHC and EKENV products amplified well; dNTP's don't seem to be a problem in the HIV system.

KP 15226-5/86 I. P. S.

Signature Susan M. Atwood

The foregoing disclosed to me on

Witness

Problem: Day 0 Keeping

Began keeping study of taq polymerase in PCR mix solution. Using @ glycerol and HIV primers. Keeping at 3 different temperatures will be monitored; -20°C, 4°C and room temp. Taq activity assay will be performed by Dave Sharkey on the same day that I test for PCR ability.

Stock Mix made:

-20°C = freezer at UofR

4°C = fridge at UofR

Room temp = dark in room 5-8128 at UofR

10X HI SALT PCR BUFFER - pH 8.0 (100mM Mg++, 100mM Tris) batch 006 prepared

dNTP'S - Sigma 100mM prepared by LB

PRIMER 1 - EKGAG-F oligo #699 10µM
PRIMER 2 - EKGAG-R oligo #776 10µM
PRIMER 3 - EKENY-F oligo #700 10µM
PRIMER 4 - EKENY-R oligo #775 10µM

KODAK TAQ - batch 3B rebuild prepare by SA

REAGENT	AMT/TUBE (µl)	# OF TUBES	STOCK AMT (µl)
10X PCR BUFFER	25	52	1300
dNTP's	15	52	780
PRIMER 1	25	52	1300
PRIMER 2	25	52	1300
PRIMER 3	25	52	1300
PRIMER 4	25	52	1300
GLYCEROL (7.5%)	18.75	52	975
TE BUFFER	56.25	52	2925
		TOTAL	11180

ALIQUOT 215µL INTO THREE TUBES. TO EACH OF THESE TUBES ADD 10µL OF 40 UNITS/µL TAQ GIVEN TO DAVE SHARKEY AND STORED ONE TUBE EACH AT -20°C, 4°C, AND ROOM TEMP. TO THE REMAINING MIX ADD 490µL OF 4 UNITS/µL TAQ. ALIQUOT 225µL INTO 48 TUBES. 3 TUBES GIVEN TO DAVE SHARKEY. STORE 8 TUBES AT ROOM TEMP., 17 TUBES AT 4°C, AND 18 TUBES AT -20°C. BEFORE RUNNING PCR, ADD 25µL OF TARGET TO EACH TUBE.

TEST DATES:
DAY 0 = 11/9/90
1 WEEK = 11/16/90
1 MONTH = 12/10/90
2 MONTHS = 1/9/91
3 MONTHS = 2/8/91
4 MONTHS = 3/15/91
6 MONTHS = 4/15/91

1µl of 2.26% thimerisol was added to each tube to be stored at room temp - prevents bacterial growth.

Susan M. Atwood

The foregoing disclosed to me on.

Witness

KP 15226-5/86 L.P.S.

RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

Notebook No. BB1001

Date

Problem: Day 0 Keeping

Initial tag activity assay:

smpl	raw cpm	cpm-bkg	dpm	nmol dA	U/ml	U/ml*dil'n	smpl	U/ml	Tot U
1	302.5	41.50	189	0.000	0.043	0.04	No Enzyme	0.00	
2	8240.0	7979.00	36268	0.082	8.258	165.17			
3	8172.5	7911.50	35961	0.081	8.188	163.77	22°C/40U	162.85	36.8
4	8795.0	8534.00	38791	0.087	8.833	176.66	4°C/40U	169.04	38.0
5	10466.5	10205.50	46389	0.104	10.563	2112.56	-20°C/40U	176.50	39.7
6	11428.5	11167.50	50761	0.114	11.558	2311.70			
7	11320.0	11059.00	50268	0.113	11.446	2289.24	22°C/400U	2211.56	499.8
8	219.5	-41.50	-189	0.000	-0.043	-0.04	4°C/400U	2373.96	534.1
9	8016.0	7755.00	35250	0.079	8.027	160.53	-20°C/400U	2296.33	516.7
10	8682.0	8421.00	38277	0.086	8.716	174.32			
11	8780.0	8519.00	38723	0.087	8.817	176.35			
12	11423.0	11162.00	50736	0.114	11.553	2310.56			
13	12030.0	11769.00	53495	0.120	12.181	2436.21			
14	11388.5	11127.50	50580	0.114	11.517	2303.42			
15	23.0						CPM	DPM	uCi
16	28.0	bkg=	261.000			Liq =	16547328.0	42429046.2	13.82
17	977.5	=	samples 1,8			Flt =	118907.0	540486.4	0.18
18	4481.5					Tot =	16666235.0	42969532.5	
							238089.1		
							53.6		

Ran this initial mix made right away in 4 blast pouches. Added 25 ul of #C018E5 target to each tube, then loaded them into blank pouches.

Pouches were run on Processor #4 with the following profile: preheat = 96°C for 10 sec

then 40 cycles of

96°C for 30 sec

70°C for 60 sec

target placed at -20°C for storage

KP 15226-6/86 I. P. S.

Signature Susan M. Atwood

Notebook No. BB1001

RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

Date

Problem: Day 0 Keeping

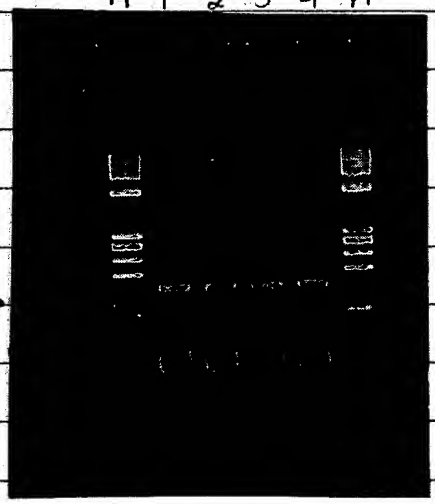
Pouches were loaded as follows:

position	serial #	
2	195074	(1)
3	195073*	(2)
6	195075	(3)
7	195076	(4)

* = PCR burst seal failure
leakage during processing

Results

Contents of PCR blister were removed into a tube and run on an EtBr stained 4% agarose gel
3ul tracking dye + 12 ul sample → load well w/ 12 ul



Marker = ΦX174 Hae III digest

Surecell Results

sample	AA7565-141 GAG	AA7565-142 ENV	AA7565-143 LTR
1	6.5/6	8/7.5	0/0
2	7/6	8.5/8	0/0
3	7/8.5	7.5/9	0/0
4	7.5/7.5	8.5/8.5	0/0

beads at 0.25%

KP 15226-5/86 L.P.S.

Signature Susan M Atwood

The foregoing disclosed to me on.

Witness

EXHIBIT B

RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

Notebook No. BB1001

Date

Problem: HIV Oligo Preparation

Obtained primers from L. Kunzelsauer - diluted to 10uM in batch 006 TE buffer Aliquotted into 400 ul volumes and stored at -20°C

CODE NAME: EKGAG-F #976 **TRITYL:** OFF
PURPOSE: HIV primer
SEQUENCE: AGT GGG GGG ACA TCA AGC AAGC CAT GCA A
U= [redacted]

LENGTH: 28

BASE COMP:	MW:(grams)	TOTAL MW:
A= 9	331.2	2980.8
C= 6	307.2	1843.2
G= 10	347.2	3472
T= 3	322.2	966.6
		9262.6

O.D: 20 (1 OD=33ug)

ug DNA: 660

Tm = 73.36

pmoles DNA: 71,254.29

CODE NAME: EKGAG-R **TRITYL:** = Biotin
PURPOSE: HIV primer
SEQUENCE: TTC CTG CTA TGT CAC TTC CCC TTG GTT C
U= 5'ADTS

LENGTH: 28

BASE COMP:	MW:(grams)	TOTAL MW:
A= 2	331.2	662.4
C= 10	307.2	3072
G= 4	347.2	1388.8
T= 12	322.2	3866.4
		8989.6

O.D: 25 (1 OD=33ug)

ug DNA: 825

pmoles DNA: 91,772.72

Tm = 70.79

[STOCK]uM: 10

RESUSPEND IN: (ul) 9,177.27

[STOCK]uM: 10

RESUSPEND IN: (ul) 7,125.43

The foregoing disclosed to me on

Susan M Atwood

Witness

RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

Notebook No. BB 1001

Date

Problem: HIV Oligo Preparation

CODE NAME: EKENV-F #977 **TRITYL:** OFF
PURPOSE: HIV primer
SEQUENCE: GGT TCT TGG GAG CAG CAG GAA GCA CTA T
U= [redacted]

LENGTH: 28

BASE COMP:	MW:(grams)	TOTAL MW:
A= 7	331.2	2318.4
C= 5	307.2	1536
G= 10	347.2	3472
T= 6	322.2	1933.2
		9259.6

O.D: 20 (1 OD=33ug)

ug DNA: 660

pmoles DNA: 71,277.38

Tm = 72.07

[STOCK]uM: 10

RESUSPEND IN: (ul) 7,127.74

CODE NAME: EKENV-R #978 **TRITYL:** BIOTIN
PURPOSE: HIV primer
SEQUENCE: CTT GAT GCC CCA GAC TGT GAG TTG CAA C
U= 5'ADTS

LENGTH: 28

BASE COMP:	MW:(grams)	TOTAL MW:
A= 6	331.2	1987.2
C= 8	307.2	2457.6
G= 7	347.2	2430.4
T= 7	322.2	2255.4
		9130.6

O.D: 20 (1 OD=33ug)

ug DNA: 660

pmoles DNA: 72,284.41

Tm = 72.07

[STOCK]uM: 10

RESUSPEND IN: (ul) 7,228.44

Signature Susan M. Atwood

The foregoing disclosed to me on.

Witness

EXHIBIT C

CONFIDENTIAL INFORMATION
CONTROLLED DISTRIBUTION
RECLASSIFY _____

RPT.ACC.NO. 249038H

DATE

THIS COPY FOR:

TECHNICAL REPORT

DIVISION
Life Sciences

KODAK RESEARCH LABORATORIES
ROCHESTER, N.Y.

TITLE
STUDIES OF DNA DENATURATION AND RENATURATION IN POLYMERASE CHAIN REACTION BUFFER: PART V. T_m'S OF COMMONLY USED PRIMERS, AND RELATIONSHIP TO AMPLIFICATION YIELDS IN PCR

AUTHORS

A. R. Mack and J.W.H. Sutherland

CONTRIBUTORS

Dr. John Findlay and
Mrs. Marlene King

ABSTRACT

T_m's in PCR buffer have been measured for a variety of primers and probes currently used on the AIDS project. The T_m's for the AIDs primers SK38 and 39 (66°C and 65.5°C) are not very different from those for beta-globin primers PC03 and PC04 (T_m's = 64.5 and 67°C, respectively).

Using a basis set of these 11 oligomers for T_m calculations, the following theoretical relationship between % guanine and cyctosine content as well as number of base pairs, N, was determined:

$$T_m(°C) = 66.5 + 0.36\,(\%G+C) - 384/N \ .$$

Although similar in functional form, this relationship differs quantitatively from analogous expressions reported in the literature.

Actual melting transitions generally were found to be in reasonable agreement with an all-or-none (i.e., thermodynamic) model using two adjustable parameters, viz., the enthalpy and entropy changes of melting. This suggests that at any given temperature these short oligonucleotides and their complements existed in equilibrium between melted and annealed forms. Hence, partially melted species did not appear to exist in detectable amounts.

Most of the primers examined here have T_m's which are less than the extension temperature of 70°C. When the yields obtained using these primers in PCR were supplemented with the T_m's measured here, it became apparent that good PCR yields required annealing at temperatures at or below primer T_m's. Respectable yields could be achieved with extension temperatures at or above T_m, but in this case most of the extension probably took place during the annealing stage. This suggests that we should either (1) lengthen the primers to raise their T_m's above the extension temperature or (2) perform two-temperature PCR's, with the lower temperature at or below the T_m of the existing (unlengthened) primers.

APPENDIX - A Test of The Regression Equation Using Additional Oligonucleotides

APPENDIX - A Test of The Regression Equation Using Additional Oligonucleotides

Following the completion of the work described in the previous text, the regression equation obtained therein (Eq. 1) was used to predict the T_m of 10 additional oligonucleotides which were not contained in the original basis set described in Table 1. The length, composition, and measured T_m's of the additional oligonucleotides is included in Table A2 . The residuals ($T_{m\ calc}$- $T_{m\ exptl}$) are given in Table A1(below):

Table A1: A Test of The Regression Equation (1)

oligonucleotide	Residual (°C)
ARM-8	0.7
ARM-9	1.0
ARM-10	0.0
ARM-11	-1.0
ARM-12	1.1
ARM-6	-0.4
IBI	-2.1
FOBS-3	-0.5
PC03-4	1.8
PC04-4	-2.5

It can be seen that the predictions are generally fairly good (the average residual being only -0.2°C).

This data was then pooled with that of Table 1, generating a pooled set of 21 oligonucleotides. The modified regression equation for this new set:

$$T_{m\ calc} = 67.5 + 0.34(\%G+C) - 395/N \qquad (A\text{-}1)$$

differed only slightly from Eq. (1):

$$T_{m\ calc} = 66.5 + 0.36(\%G+C) - 384/N \ .$$

Residuals and fits are shown in Table A-2 and Figs. A-1 and A-2.

Notebook No. AA9223

RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

Date

Problem: Coamplification of Multiple Primers

Send to: Dan
From: Thomas Cummins
Subject: Patents

These are important patent items:

1. Common Tms for coamplification of two or more primer sets using PCR.

Outline. When applying two temperature PCR whereby the annealing and extension temperature are combined, it is important that multiple primer sets are structured so that the Tm of the primers are equivalent. Tm is dictated primarily by primer length and G / C content. Equal Tms (w/in 1 - 3 degrees) will ensure thatmultiple primer pairs anneal at roughly the same efficiency so that the overall efficiency of the amplification reaction is not altered for any one of the multiple primers. This ensures that the sensitivity of the primers is equal.

advantages....Fast PCR = better specificity, faster test time. Common Tms for coamplified primers = allows multiple sets of primers to be coamplified in a single reaction using two temperature PCR ensuring equivalent amplification efficiencys for all complified primers.

efficiencys for all complified primers.

Example:

Targets HIV gag gene and env gene:

Primers = gag primer = Tm 68 C..... env primer = Tm = 58 C...if these primer sets are coamplified using 2 temp PCR, where the annealing / extension temperature is 67 C, only the gag primer will amplify because the temperature is well above the melting point of the env primer and the primer will not bind at all to the target sequence or bind at a much lower efficiency than the gag primer leading to a compromized amplification.

By bringin the env Tm to 68 C (similar to the gag primer) both sets can now be amplified at the same efficiency at the single temperature of 67 C.

Thanks, Tom

Signature

The foregoing disclosed to me on.

Witness

RESEARCH / DEVELOPMENT

EASTMAN KODAK COMPANY

Notebook No. #A9223

Date ~~AA9223~~

Problem: Similar Tms for the codetection of amplified

E04

SEND A NOTE

Send to: Dan
From: Thomas Cummins
Subject: Patent

2. Commen probe Tms for the codetection of two or more amplified nucleic acid seguences following the polymerase chain reaction.

Outline: When detection two or more amplified nucleic acid sequences (products) following coamplification using the polymerase chain reaction, it is an advantage to have the probe/product complex for each primer set constructed so that the Tms are roughly the same (w/in 1 - 3 degrees). Similar Tms will thus allow a single wash stringency (temperature and ionic strength) to be used during the detection chemistry. This is desirable in a system where a single chemistry is to be used for the detection of multiple products.

Thanks, Tom

Signature

The foregoing disclosed to me on

Witness